

Commentary

Silencing Bcl-X_L in Cancer Therapy

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Commentary to:

Enhancing TRAIL-Induced Apoptosis by Bcl-X_L siRNA

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There are two main signaling pathways that lead to apoptosis: an “extrinsic” and an “intrinsic” pathway. The Tumor Necrosis Factor (TNF)-related apoptosis-inducing ligand (TRAIL) triggers the extrinsic pathway and is a promising agent for development as a cancer therapeutic¹ because it appears to specifically kill transformed and cancer cells, whereas most normal cells appear to be resistant to TRAIL.^{2,3} TRAIL binding to either of two pro-apoptotic TRAIL death receptors, TRAIL R1 (DR4)⁴ or TRAIL R2 (KILLER/DR5),⁵ induces the formation of a death-inducing signaling complex (DISC), which consists of one of the TRAIL receptors, the adaptor protein FADD,⁶ and an initiator caspase, procaspase-8. Once the DISC is formed, procaspase-8 is auto-processed and activated by induced proximity.⁷ The activated caspase-8 can directly activate the executioner caspase, caspase-3, without the involvement of the mitochondrial pathway (type I pathway), or it can cleave Bid. Truncated cleaved Bid (tBid) exposes a new amino-terminal glycine that undergoes post-translational myristoylation.⁸ The myristoylated Bid translocates to the mitochondria where tBid inserts into the membrane⁹ followed by Bax translocation, Bak oligomerization, and cytochrome c release (type II pathway).¹⁰

The intrinsic pathway in response to DNA damage, such as that caused by radio- and chemotherapeutic agents^{11,12} involves the participation of mitochondria, which release cytochrome c.⁸ The released cytosolic cytochrome c induces oligomerization of Apaf-1 and recruitment of procaspase-9 into a large complex known as the apoptosome.¹³ After activation in the apoptosome, caspase-9 activates the effector caspases,^{13,14} thereby initiating apoptosis.

The Bcl-2 family proteins are the major regulators of mitochondrial apoptotic homeostasis.¹⁵ Several members of the Bcl-2 family (including Bcl-2, Bcl-X_L, MCL-1, A1 and BAG-1) promote survival while the other members (including Bcl-X_S, Bad, Bax, and Bak) promote cell death. The relative ratios of these pro- and anti-apoptotic members (i.e., homodimers:heterodimers) of the Bcl-2 family, rather than the expression level of any single Bcl-2 family protein, have been shown to determine the ultimate apoptotic sensitivity or resistance of cells to diverse stimuli.¹⁶ Anti-apoptotic proteins including Bcl-2 or Bcl-X_L are frequently overexpressed in human tumors,¹⁷ thereby blocking death signal propagation through mitochondria.¹⁸ Accordingly, targeted knock-down or silencing of the anti-apoptotic Bcl-2 family has potential to facilitate cancer cell apoptosis induced by various apoptotic stimuli. Until recently, the repression of these genes has been mediated by anti-sense oligonucleotides. The oligonucleotides targeting Bcl-X_L could sensitize tumor cells to various chemotherapeutic agents,¹⁹ agonistic anti-FAS Ab,²⁰ or to TRAIL.^{21,22} Recently small interfering RNAs (siRNAs) that are 21- to 23 nucleotides of dsRNA²³ have emerged for repression of these genes. For example, Bcl-X_L protein expression was silenced by Bcl-X_L-specific synthetic siRNA in 5-FU-resistant cancer cells and proliferation of the cells became inhibited by 5-FU.²⁴

In the present study, Zhu et al show that the combination of Bcl-X_L siRNA and TRAIL inhibited cell proliferation and sensitized TRAIL-induced apoptosis in human cancer cells with both acquired (DLD1-TRAIL/R, a TRAIL-resistant derivative selected from human colon cancer DLD-1 parental cells) and intrinsic (human ovarian cancer SKOV3 cells, which express high levels of Bcl-X_L and have K441R polymorphism in the death domain of DR4²⁵) TRAIL resistance. The results show that although there is detectable cytochrome-c release into cytosol by Bcl-X_L siRNA alone, TRAIL alone, or the combination of Bcl-X_L siRNA and TRAIL in both cell lines, there was no release of Smac/DIABLO^{26,27} from mitochondria in any of the treatment groups. Smac/DIABLO is normally localized to mitochondria but is released into the cytosol during the early stages of apoptosis, where it promotes caspase activity by inhibiting IAPs, particularly the X-linked inhibitor of apoptosis (XIAP).^{28,29} Overexpression of Bcl-X_L prevented mitochondrial release of Smac/DIABLO and subsequent inactivation of the XIAP protein.³⁰ Overexpression of the IAP family proteins is associated with poor responsiveness to apoptosis-inducing

therapies.^{31,32} Therefore, it is possible that the outcome of treatment using the combination of Bcl-X_L siRNA and TRAIL may be affected by the expression level of endogenous XIAP in cancer cells.

To have broad applications for cancer therapy, the downregulation of Bcl-X_L should have minimal cytotoxicity toward normal cells. Although severe clinical side effects related to the transient downregulation of Bcl-X_L or Bcl-2 in normal cells and other normal tissues have not been reported, there was a report that normal cells underwent apoptosis upon treatment with Bcl-X_L or Bcl-2/Bcl-X_L anti-sense oligonucleotides.³³ To avoid this potential side effect, Bcl-X_L siRNA should either be delivered to the target cells in a tumor-specific manner or the target Bcl-X_L should be specifically expressed in the tumors being treated. Recently, there have been reports on schemes that could be used to express siRNAs in a tissue specific manner.^{34,35} By modifying these schemes, Bcl-X_L siRNA could be expressed specifically in cancer cells and thus, unwanted cytotoxicity toward normal cells could be circumvented and therapeutic sensitization of cancer cells toward TRAIL or other cancer chemotherapeutics could be achieved.

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